84-2 - Unscheduled DNA Synthesis in Rat Hepatocytes
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DATA EVALUATION REPORT

Study Type: DNA Repair Assay in Rat Hepatocytes **TOX. CHEM. NO.:** 2980

Accession No.: 403888-13 **MRID NO.:**

Test Material: CGA-154281 Technical (Batch No. FL 870211; 94% Purity)

Synonyms:

Study Number (s): 871079

Sponsor: CIBA-GEIGY Corp.

Testing Facility: Experimental Pathology, CIBA-GEIGY Limited, Basle, Switzerland

Title of Report: Autoradiographic DNA Repair Test on Rat Hepatocytes

Author(s): T. Hertner, I. Hunziker, M. Maurer and K. Mennle

Report Issued: July 2, 1987

Conclusions:

CGA-154281 Technical did not cause DNA damage or inducible repair in rat hepatocyte unscheduled DNA synthesis at the concentrations tested (0.1 through 20 ug/ml).

Concentrations tested: 0.1, 0.5, 2, 4, 6, 8, 10 and 20 ug/ml in the 1st trial; 0.1, 0.5, 2, 4, 6, 8, 10 and 20 ug/ml in the 2nd trial

Classification of Data: Acceptable

Title of Study: Autoradiographic DNA-Repair Test on Rat Hepatocytes
(Test Material: CGA-154281 Technical)
Ciba-Geigy Limited Experimental Pathology Laboratory
Test No. 871079

I. Materials and Methods:

1. Test Materials

The test compound, CGA-154281 Technical (Batch No. FL 870211; 94% Purity), dissolved in DMSO was used in this study. 4-Aminobiphenyl (25 and 50 uM) was used as the positive control.

2. Medium

Williams' medium E containing 10% fetal bovine serum, 100 U/ml penicillin, 100 ug/ml streptomycin and 2.5 ug/ml amphotericin.

3. Indicator Cells

Primary hepatocytes were isolated from adult male rats (Tif.RAIf(SPF); 170-350 g) by in situ-collagenase perfusion according to the method described by M.N. Berry and D.S. Friend (J. Cell Biol. 43: 506-520, 1969) as modified by L.R. Schwartz et al (Eur. J. Biochem. 94: 617-622, 1979). Monolayer cultures were established on gelatinized THERMANOX coverslips in culture plates for initiation of the UDS assay. All cultures were maintained as monolayers at about 37°C in a humidified atmosphere with 5% CO_2.

4. Toxicity Test

A toxicity test was performed to determine the highest concentration to be used in the DNA-Repair test. Attached primary cells were exposed to a wide range of concentrations of CGA-154281 Technical (7.5, 15, 30, 60, 80, 100, 200, 400, 600, 800 and 1000 ug/ml) for 16-18 hours. After exposure, cells were washed with BSS, stained with Trypan-Blue solution (0.2%) and the percentage of unstained cells evaluated by counting 100 cells.

5. UDS Assay

The freshly isolated rat liver cells attached on coverslips (4×10^5 viable cells) were used. Following the addition of CGA-154281 Technical and ^{3}H-thymidine (8 uCi/ml) in the culture compartments with 2 ml of medium, the culture compartments were incubated for 16-18 hours at 37°C. After incubation, the treated cultures were washed with BSS, swelled with 1% sodium citrate and fixed with ethanol/acetic acid (3:1). The coverslips were mounted on microscope slides and prepared for autoradiography. The exposure time was 6 days. The autoradiographs were stained with hematoxylin-eosine.

6. Grain Counting

Counting of silver grains over the nuclei and cytoplasm of the hepatocytes was carried out with the aid of an electronic counter (ARTEK Model 982) attached to a microscope at a magnification of 2000 X. From each of the treatment groups and from the positive and the negative controls, 150 nuclei in altogether three slides (50 cells/slide) were scored. The incorporation of radioactive material in the cytoplasm was determined by counting the silver grains in three nucleus-equivalent areas of cytoplasm adjacent to the nucleus. The net values were calculated by subtracting the average grain count over the cytoplasm from the total over the nuclei.

7. Evaluation Criteria

The test compound is generally considered to be active in the DNA-Repair test, if one of the following conditions is met:

(a) The mean number of silver grains per nucleus in relation to the vehicle control is more than doubled at any concentration.

(b) The mean number of silver grains per nucleus in relation to the vehicle control shows a concentration dependent increase and at least at one concentration a statistically significant increase in comparing with the vehicle control is demonstrated ($P<0.05$).

(c) The percentage of nuclei with a number of silver grains greater than the one calculated from the distribution at the vehicle control is 10% or more.

II. Reported Results:

1. Preliminary Toxicity Test (Table 1 attached)

The cells were exposed to 7.5, 15, 30, 60, 80, 100, 200, 400, 600, 800 and 1000 ug/ml of CGA-154281 Technical, resulting in a percent viable cell range of 43% at 30 ug/ml to 86% at 7.5 ug/ml. Precipitation was visible in the culture medium at 200 ug/ml and above. Based on these data, 30 ug/ml was selected as the highest dose for the UDS assay.

2. UDS Assay

(a) Original DNA-Repair Test (Tables 2 and 3 attached)

The original DNA-Repair test was carried out with concentrations of 0.3, 2, 5, 10, 20 and 30 ug/ml. Comparison of the mean number of net grains per nucleus in the vehicle control (-0.03) and after treatment with six concentrations of CGA-154281 Technical revealed no dose-related increase in this study. However, at a concentration of 5 ug/ml a net value of 3.47 was obtained. Also, with the exception of 5 ug/ml dose group, no meaningful difference in the percentage distribution range of siver grains per nucleus was found between the treated groups and vehicle control (Nuclei with 8 silver grains: Vehicle control 53.3%, 5 ug/ml 66%; 10 ug/ml 21.3%; 20 ug/ml 2.7%; 30 ug/ml 0%).

(b) Confirmatory DNA-Repair Test (Tables 4 and 5 attached)

In the confirmatory experiment, concentrations of 0.1, 0.5, 2, 4, 6, 8, 10 and 20 ug/ml were used. Comparison of the mean number of net grains per nucleus in the vehicle control and the CGA-154281-treated groups revealed no marked differences (Net grains/nucleus: Vehicle control 1.67; 0.1 ug/ml 0.54; 0.5 ug/ml -1.49; 2 ug/ml 0.60; 4 ug/ml 0.08; 6 ug/ml 0.37; 8 ug/ml -0.36; 10 ug/ml -0.46). In addition, there was no significant difference ($P<0.05$) in the percentage distribution range of silver grains per nucleus found between the treated groups and vehicle control (Nuclei with 8 silver grains: Vehicle control 19.3%; 2 ug/ml 8%; 4 ug/ml 7.3%; 6 ug/ml 12%; 8 ug/ml 10.7%; 10 ug/ml 13.3%).

III. Evaluation and Recommendation:

1. The positive control, 4-ABP (25 and 50 uM), induced significant increase in the net nuclear count per nucleus exceeding 8.87 and 9.73 in the 1st trial and 2nd trial respectively. These results indicate that the cell population employed was responsive and the methodology was adequate for the detection of UDS in rat hepatocytes.

2. The test material has been tested to cytotoxicity level (i.e., 30 ug/ml). Duplicate tests (original and confirmatory) have been employed to measure UDS in rat hepatocytes using the autoradiographic technique for this study. The mean net nuclear grain counts were determined from the triplicate coverslips (50 nuclei/coverslip).

3. The nuclear labeling in the negative (solvent) control was found within the normal range of net nuclear grain count per nucleus (DMSO control: -0.03 to 1.67).

4. Under the test conditions reported, the test material failed to induce any significant changes ($P<0.05$) in the nuclear labeling (net nuclear counts/nucleus) of rat hepatocytes at the dose levels tested (0.1 through 20 ug/ml). Therefore, the test material was inactive in the unscheduled DNA synthesis in primary rat hepatocytes. The study is acceptable.

4-(Dichloroacetyl)-3,4-Dihydro-3-Methyl-
2H-1,4-Benzoxazine

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